SPECIAL REPORT

Gene-Panel Sequencing and the Prediction of Breast-Cancer Risk

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Advances in sequencing technology have made multigene testing, or "panel testing," a practical option when looking for genetic variants that may be associated with a risk of breast cancer. In June 2013, the U.S. Supreme Court[1] invalidated specific claims made by Myriad Genetics with respect to the patenting of the genomic DNA sequence of *BRCA1* and *BRCA2*. Other companies immediately began to offer panel tests for breast cancer genes that included *BRCA1* and *BRCA2*. The subsequent flourishing of gene-panel testing services (Table 1, and Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) has generated much interest both within the clinical genetics community and in the popular press.[2] These panels cover a total of more than 100 genes, and breast cancer is specifically mentioned as an indication for 21 of these genes. However, the fact that the technology is available does not necessarily mean that such tests are appropriate or desirable.

According to the framework proposed by the ACCE (established by the Centers for Disease Control and Prevention), genetic tests should be evaluated on the basis of the four criteria from which the name ACCE is derived: analytical validity, clinical validity, clinical utility, and ethical, legal, and social issues.[3] Analytical validity refers to the degree of accuracy with which a test detects the presence or absence of a mutation. Here, however, we focus on the key question of clinical validity: Are the variants the test is intended to identify associated with disease risk, and are these risks well quantified? The validity of the risk estimates is a key determinant of the clinical utility of panel testing, which in turn should inform decisions regarding the adoption of the testing in clinical practice. We do not consider in detail who should undergo testing, what level of risk is associated with any given variant that might be considered clinically useful, or how that risk might be managed. However, broadly similar guidelines for managing the care of women with a family history of breast cancer exist in several countries (Table 2). These guidelines are based on the stratification of patients according to levels of risk and provide guidance on the identification of women to whom screening (by means of mammography or magnetic resonance imaging), risk-reducing medication, and risk-reducing surgery should be offered. These recommendations could be modified to reflect the identification of risk variants through the use of gene-panel testing. Whatever the recommendations for the management of care, the underpinnings of the guidelines should be based on reliable estimates of the risk of cancer.

Before these guidelines are developed, the appropriateness of the tests themselves needs to be considered. The determination of analytical validity for laboratory-developed diagnostic tests falls under the remit of the Clinical Laboratory Improvement Amendments (CLIA) of 1988, but neither clinical validity nor clinical utility is part of the assessment process. Therefore, whereas new drugs without clinical utility will not be approved by the Food and Drug Administration (FDA), gene-panel tests can be adopted without

Table 1. Examples of Multigene Testing Panels for Breast Cancer.*

Company	Test	Website	Genes Included†
Ambry Genetics	BreastNext	www.ambrygen.com/tests/breastnext	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, TP53*
BreastHealth UK	BreastGene	www.breasthealthuk.com/screening-services/genetic-testing/breastgene	*ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, STK11, TP53*
Centogene	Breast Ovarian Cancer Panel	www.centogene.com/centogene/centogene-test-catalogue.php	*ATM, BARD1, BRIP1, CDH1, CHEK2, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, XRCC2*
Emory Genetics Laboratory	High Risk Breast Cancer Panel	http://geneticslab.emory.edu/tests/MM201	*PTEN, STK11, TP53*
Fulgent Diagnostics	Breast Ovarian Cancer NGS Panel	http://fulgentdiagnostics.com/test/breast-ovarian-cancer-ngs-panel/	*APC, ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNB1, EPCAM, FANCC, HOXB13, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PALLD, PMS2, PTEN, RAD50, RAD51, RAD51C, RAD51D, SMAD4, STK11, TP53, VHL, XRCC2, XRCC3*
GeneDx	OncoGeneDx	www.genedx.com/test-catalog/available-tests/breastovarian-cancer-panel	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53, XRCC2*
Illumina	TruSight Cancer	www.illumina.com/clinical/translational_genomics/panels/kits.html	94 Genes plus 287 SNPs reported to be associated with risk of breast cancer
Invitae	Hereditary Breast Cancer, High-Risk Panel	www.invitae.com/en/physician/panel-detail/PNL0009/	*BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53*
Myriad Genetics†	myRisk	www.myriad.com/products-services/hereditary-cancers/myrisk-hereditary-cancer/	*ATM, BARD1, BRCA2, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C,* STK11, *TP53*
CD Genomics	Genetic Testing for the Cancer Susceptibility	www.cd-genomics.com/Genetic-Testing-for-the-Cancer-Susceptibility.html	Not specified
University of Washington†	BROCA – Cancer Risk Panel	http://web.labmed.washington.edu/tests/genetics/BROCA	*AKT1, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, GEN1, MRE11A, MUTYH, NBN, PALB2, PIK3CA, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2*

* SNP denotes single-nucleotide polymorphism.
† For Myriad Genetics and the University of Washington, only genes for which breast-cancer risk is given as an indication are listed. For a complete list, see Table S1 in the Supplementary Appendix. In several cases, the panels include additional genes, and several companies also offer larger panels. Thus, even if the primary purpose of the test is prediction of the risk of breast cancer, results will often be available (and need to be interpreted) for a larger set of genes than those listed here.

any review of data regarding their clinical utility.[5,6] Recent commentaries have suggested ways in which the FDA might become involved in the approval of genomic tests.[7,8] Although we acknowledge the enormity of the task, we propose that a genomic test should not be offered until its clinical validity has been established. We consider below some of the key issues that need to be addressed. Others have argued that establishing clinical validity is a postmarketing pursuit,[8] but we believe that failing to require the clinical validation of genomic tests before they are submitted for regulatory approval is likely to lead to substantial misuse of the technology.

Common versus Rare Variants.

Approximately 100 independent common variants (consisting primarily of single-nucleotide polymorphisms [SNPs]) associated with breast-cancer risk have been identified through large-scale genotyping studies. These variants typically have minor allele frequencies higher than 1%, and all confer risks that are less than 1.5 times as high as those in the general population; almost all these polymorphisms occur in noncoding sequences. Some commercial genetic panels include a subset of these SNPs. Thus, at present, there is a reasonably clear distinction between SNPs that confer a small increased susceptibility to breast cancer and variants that confer a moderate-to-high susceptibility as identified through sequencing. However, some sequence variants located in genes classified as conferring a high or moderate risk confer risks that fall below the threshold for moderate risk (i.e., two times as high as that in the general population). Examples include *BRCA2* p.Lys3326Ter and *CHEK2* p.Ile157Thr. Use of the term "low risk" for variants conferring a risk that is less than moderate is widespread, but it is not a particularly helpful term for counseling purposes, since carriers of such variants are still at an elevated level of risk.

KEY ISSUES AND GENERAL PRINCIPLES

Several key questions must be addressed in order to establish clinical validity. First, are variants in the gene associated with breast-cancer risk? Second, which variants, or classes of variants, are associated with risk? Third, what is the magnitude of those risks? Fourth, what methods have been used to estimate those risks? We will concentrate on the genes in which rare variants have been proposed to confer a moderate or high risk of breast cancer. For the purpose of this review, we define moderate risk as a risk of breast cancer, defined in terms of disease incidence, that is two to four times as high as that in the general population and high risk as an incidence that is more than four times as high.[9] We leave aside the separate question of risk prediction in which profiling based on the genotyping of common polymorphisms is used (see box). We will restrict our attention to the prediction of risk in women unaffected by breast cancer, although somewhat analogous issues apply to testing in affected women. We focus on the question of breast-cancer risk, but similar considerations apply to other cancers. Indeed, some of the genes considered here also confer a predisposition to ovarian cancer, pancreatic cancer, and other cancers, and some of the available panels also include genes putatively involved in a wider range of cancers (Table 3, and Table S2 in the Supplementary Appendix). We leave aside the use of panel testing for the identification of cancer syndromes and for the management of disease in women who have cancer.

TYPES OF GENETIC VARIANTS

Most panel testing involves identifying the coding sequences and splice junctions of the genes of interest, often in combination with alternative methods used for the detection of large genomic rearrangements.[35] Most of the variants identified are single-base substitutions and small insertions or deletions (indels). We refer to all nonsense substitutions, frameshift indels, and variants affecting splicing as protein-truncating variants. For the large majority of genes, most of the evidence on breast-cancer risk relates to protein-truncating variants assumed to result in loss of function.

STATISTICAL SIGNIFICANCE AND BURDEN TESTS

It is important to establish stringent levels of statistical significance. Although it would be ideal to have specific evidence for every variant detected, most variants for which there is a suspicion of association with a high risk of disease are rare, and the sample sizes required to establish allele-specific associations with risk are so large as to make the task infeasible. Consequently, some form of burden testing is frequently used in which the association between carrying any variant in a specific class and the risk of disease is evaluated. A potential problem with this method is that it does not indicate whether any specific variant identified is associated with disease. It is often assumed that all protein-truncating variants are equally pathogenic; however, all such variants do not confer the same risks. For missense variants, the situation is even more problematic.

STRENGTH OF STATISTICAL EVIDENCE FOR ASSOCIATION

The issue of what constitutes appropriate levels of significance for targeted sequencing has not been extensively discussed. An exomewide sig-

Table 2. Screening and Treatment Guidelines for Carriers of *BRCA1* or *BRCA2* Mutations.*

Type of Screening or Therapy	NCCN (United States)	NICE (United Kingdom)†	GC-HBOC– (Germany)	eviQ Cancer Treatments Online (Australia)	IKNL–KiMS (Netherlands)
Mammography	Recommended annually at 30–75 yr of age, or younger if woman in family has received breast-cancer diagnosis before 25 yr of age and MRI is not available	Recommended for consideration annually at 30–39 yr of age; recommended once yearly at 40–69 yr of age and every 3 yr at ≥70 yr of age	Recommended every 1–2 yr at 49–69 yr of age if breast density classified as ACR 1 or 2, with ultrasonography twice yearly‡	For *BRCA1* carriers, recommended annually at 30–50 yr of age, with or without ultrasonography For *BRCA2* carriers, recommended annually at 30–50 yr of age, with or without ultrasonography; at >50 yr of age, mammography recommended annually, with or without ultrasonography, with clinical breast examination; if diagnosis in family member <35 yr of age, may recommend individualized schedule	Recommended annually; because risk of radiation-induced tumors is greater in young women, first mammogram recommended at 30 yr of age
MRI	Recommended annually at 25–75 yr of age, but earlier if younger age of onset in any family member	Recommended annually at 30–49 yr of age unless breast density is high, in which case should be continued until 70 yr of age	Recommended annually at 25–69 yr of age if breast density is classified as ACR >1	For *BRCA1* carriers, recommended annually at 30–50 yr of age, with or without ultrasonography For *BRCA2* carriers, recommended annually at 30–50 yr of age, with or without ultrasonography; at >50 yr of age, mammography recommended annually, with or without ultrasonography, with clinical breast examination; if diagnosis in family member <35 yr of age, individualized schedule may be recommended	Recommended annually, starting 25 yr of age
Preventive mastectomy	No definitive guideline, but "degree of protection and risks" should be discussed	No definitive guideline, but discussions of potential benefits of surgery should take current age into account	No definitive guideline, but "degree of protection and risks" should be discussed	If performed, recommended at ≤40 yr of age	Recommended at ≥25 yr of age; <5% of patients are at risk of residual breast cancer
Preventive oophorectomy	If performed, recommended between 35 and 40 yr of age	No guideline	Salpingo-oophorectomy recommended at approximately 40 yr of age for *BRCA1* carriers and 45 yr of age for *BRCA2* carriers	If performed, recommended at ≤40 yr of age	If performed, recommended at ≥35 yr of age for *BRCA1* carriers and ≥40 yr of age for *BRCA2* carriers
Oral contraceptive	No clear directive	No clear directive	No clear directive	Combination oral contraceptive not contraindicated	No clear directive; recommended that nonsystemic form of contraception could be discussed
Chemoprevention	No clear directive	Provision of tamoxifen recommended for women at high risk of breast cancer; but *BRCA1* vs. *BRCA2* status not discussed	No guideline	Recommendation to consider with professional on individualized basis	No guideline

Hormonal therapy	No clear directive	No guideline	Not indicated for carriers of *BRCA1* or *BRCA2* unless preceded by risk-reducing salpingo-oophorectomy	If risk-reducing salpingo-oophorectomy performed before menopause, hormone-replacement therapy should be considered until onset of natural menopause	No guideline
Screening for other cancers	Prostate-cancer screening recommended for *BRCA2* mutation carriers ≥40 yr of age (consider for *BRCA1* mutation carriers)	No guideline	Prostate-cancer screening recommended for *BRCA2* mutation carriers 45–50 yr of age	Consideration should be given to annual testing for prostate-specific antigen and digital rectal examinations for prostate cancer from approximately 40 yr of age onward	No guideline

* A summary of guidelines available in the United States is provided by the National Cancer Institute; see Table 12.[4] GC-HBOC–AGO denotes German Consortium for Hereditary Breast and Ovarian Cancer–German Gynecological Oncology Group, MRI magnetic resonance imaging, NCCN National Comprehensive Cancer Network, and NICE National Institute for Health and Care Excellence.
† The NICE guidelines focus on familial breast cancer only, and they are not confined to the discussion of carriers of *BRCA* mutations.
‡ ACR denotes American College of Radiologists, with ACR 1 indicating breast-tissue involution, ACR 2 scattered fibroglandular tissue, ACR 3 heterogeneously dense parenchyma, and ACR 4 extremely dense tissue composition.

nificance level of $P<2.5\times10^{-6}$ is often used for whole-exome studies (calculated on the basis of a Bonferroni correction for approximately 20,000 genes). Since most genes associated with susceptibility to breast cancer are involved in DNA repair (a class involving fewer than 500 genes), more liberal significance levels (on the order of P<0.0001) might be appropriate for genes in this pathway. The use of Bayesian arguments leads to similar thresholds (see the Methods section in the Supplementary Appendix). Although these significance thresholds may be appropriate for a single burden test, more stringent thresholds would be required for calculations involving individual variants. A related question is the precision of the risk estimate. It is clearly undesirable to give a patient an estimate of risk that may be subject to substantial change when additional data are acquired. For the purposes of this review, we consider it to be likely that a given risk will be above (or below) a certain threshold if the 90% confidence limit on the risk estimate exceeds (or is less than) the threshold.

DEFINITION OF RISK

Our estimates are presented primarily in terms of average relative risks. We recognize that for purposes of counseling, absolute estimates of risk (projected over a few years or a lifetime) are more useful. However, most studies report estimates of relative risk rather than absolute risk, and absolute risks are more strongly influenced by risk factors for breast cancer, such as a family history of breast cancer, age at menopause, and breast density on mammography. In the case of a rare variant conferring a relative risk of 2 or 4, the corresponding absolute risks of breast cancer would be approximately 18% and 32%, respectively, by the time a patient reached 80 years of age (according to recent U.K. incidence rates),[36] in the absence of other causes of death. These risks approximately correspond to the definitions of moderate and high risk familiar to the clinical genetics community.[4]

It follows that the identification of a variant conferring a relative risk higher than 4, in the absence of any other data, can place a woman in the high-risk category. In contrast, a variant conferring a relative risk of 2 to 4 will place a woman in the high-risk category only if her risk is increased by other factors. For some genes (notably, *BRCA1*,[10] *CHEK2*,[31] and *ATM*[27]), there is

Table 3. Genes for Which an Association between Protein-Truncating Variants and Breast-Cancer Risk Has Been Established.

Gene	Magnitude of Relative Risk Associated with Truncating Variants*		Risk Associated with Missense Variants†	Estimated Relative Risk (90% CI)‡	P Value	Absolute Risk by 80 Yr of Age§	Comments	Other Associated Cancers	References
	Moderate	High				%			
BRCA1	Yes	Yes	Yes	11.4		75	Estimates are based on the BOADICEA model for a woman born in 1960	Ovary	Antoniou et al.,[10] Lee et al.,[11] Chen and Parmigiani,[12] Mavaddat et al.[13]
BRCA2	Yes	Yes	Yes	11.7		76	Estimates are based on the BOADICEA model for a woman born in 1960; p.Lys3326Ter in the carboxyl terminus is associated with a lower increase in risk	Ovary, prostate, pancreas	Antoniou et al.,[10] Lee et al.,[11] Chen and Parmigiani,[12] Mavaddat et al.[13]
TP53¶	Yes	Yes	Yes	105 (62–165)			Most published risk estimates are subject to ascertainment bias	Childhood sarcoma, adrenocortical carcinoma, brain tumors	Hisada et al.,[14] Hwang et al.[15]
PTEN	Unknown	Unknown	Yes	No reliable estimate‖			Published risk estimates are subject to ascertainment bias	Thyroid, endometrial cancer	Bubien et al.,[16] Tan et al.[17]
CDH1	Likely	Unknown	Unknown	6.6 (2.2–19.9)	0.004	53	Specific to lobular breast cancer	Diffuse gastric cancer	Pharoah et al.[18]
STK11	Unknown	Unknown	Unknown	No reliable estimate**			Published risk estimates are subject to ascertainment bias	Colon, pancreas, ovarian sex cord–stromal tumors	Hearle et al.[19]
NF1	Likely	Unlikely	Unknown	2.6 (2.1–3.2)	2.3×10^{-13}	26	Estimates are based on cohort studies of patients with neurofibromatosis type 1††	Malignant tumors of peripheral nerve sheath, brain, central nervous system	Madanikia et al.,[20] Seminog and Goldacre[21]
PALB2	Likely	Unknown	Unknown	5.3 (3.0–9.4)	4×10^{-10}	45	Estimates are based on a meta-analysis of published case–control and family studies	Pancreas	Antoniou et al.,[22] Heikkinen et al.,[23] Rahman et al.,[24] Erkko et al.[25]

ATM	Likely	Unlikely	Yes	2.8 (2.2–3.7)	5×10^{-11}	27	The p.Val2424Gly variant is associated with higher risk than truncating variants	Pancreas	Renwick et al.,[26] Thompson et al.,[27] Janin et al.,[28] Olsen et al.[29]
CHEK2	Likely	Unlikely	Yes	3.0 (2.6–3.5)	8×10^{-37}	29	Most data for truncating variants are limited to the variant c.1100delC; p.Ile157Thr is associated with an increase in risk that is 1.3 times as high as in the general population	Lung, although p.Ile157Thr is associated with reduced risk	Meijers-Heijboer et al.,[30] CHEK2 Breast Cancer Case–Control Consortium,[31] Weischer et al.,[32] Kilpivaara et al.[33]
NBN	Likely	Unlikely	Unknown	2.7 (1.9–3.7)	5×10^{-7}	23	Almost all data pertain to the c.657del5 variant in Slavic populations	Unknown	Zhang et al.[34]

* Moderate risk is defined as an average increase that is two to four times as high as that in the general population (on the basis of disease incidence) and high risk as an increase that is more than four times as high. When a quantitative analysis has been performed, "likely" indicates that the lower 90% confidence limit on the relative-risk estimate exceeds the threshold, and "unlikely" indicates that the upper 90% confidence limit on the relative-risk estimate is lower than the threshold.

† The majority of missense variants have not been shown to be associated with risk.

‡ Estimates were obtained from the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) risk model for *BRCA1* and *BRCA2*, from a single study for *TP53* and *CDH1*, and from a meta-analysis of multiple studies for the other genes (see the Methods section and Table S3 in the Supplementary Appendix for further details). Note that there is evidence that relative risk declines with age for carriers of mutations in *BRCA1*,[10] *CHEK2*,[31] and *ATM*[27] the evidence for *PALB2*[22] is weaker. These represent average relative risks for all ages and may therefore underestimate the relative risk of breast cancer for younger persons and overestimate the relative risk for older persons. The estimates relate for protein-truncating variants, except as noted (see the Methods section in the Supplementary Appendix).

§ Absolute risks are risks in the absence of other causes of death. Adjusted estimates that allow for competing mortality will be lower, especially when the risk of other cancers is high (e.g., in *BRCA1* and *BRCA2*). Unless otherwise indicated, the values shown were estimated by applying the estimated relative risks to breast-cancer incidence rates in England for 2003 through 2007.[36]

¶ Most pathogenic mutations in *TP53* are missense variants.

‖ In *PTEN* mutation carriers, relative risks of breast cancer of 39.1 (90% CI, 26.7 to 54.9) and 25.4 (90% CI, 20.6 to 30.8) have been reported in two studies.[16,17] However, estimates were based on selected families with the Cowden syndrome or related syndromes, resulting in an overestimate of risk.

** The cumulative breast-cancer risk for women with the Peutz–Jeghers syndrome has been reported to be 45% by 70 years of age (90% CI, 29–64).[19] However, this estimate did not allow for ascertainment, which would result in an overestimate of risk even in families at high risk for disease. Furthermore, the data included patients with the Peutz–Jeghers syndrome in whom no *STK11* variant had been identified.

†† Risk estimates are based on follow-up data from patients with neurofibromatosis type 1, which is caused by both truncating and missense mutations in *NF1* (although the majority are protein-truncating mutations). There are no published risk estimates for mutations in *NF1* according to mutation type.

Table 4. Study Designs for Estimating the Risks Associated with Rare Variants.

Method	Description	Advantages	Disadvantages	Example
Population-based case–control	Screening is conducted for variants in unselected cases of disease and population-matched controls	Provides direct estimates of the relative risk (odds ratio) and is not biased by other familial factors	Must be very large since variants are typically rare; biases arise if controls are not appropriately population-matched (there are large differences in allele frequency among populations)Requires that the same assay techniques are used for cases and controls to provide valid tests and estimates, typically with screening of the full coding sequences in all cases and controls; large biases may arise if only the variants identified in the cases are tested in the controls Requires that the same assay techniques are used for cases and controls to provide valid tests and estimates, typically with screening of the full coding sequences in all cases and controls; large biases may arise if only the variants identified in the cases are tested in the controls	*CHEK2*[31]
Family-based case–control	In theses case–control studies, cases are enriched by family histories	Improves power because of the higher frequency of variants in familial cases	Is subject to biased risk estimates Efforts to correct bias depend on use of additional assumptions about the modifying effects of other familial factors	*CHEK2*[30]
Kin cohort	Data on cancer occurrence in relatives of carriers in population-based series are used to estimate risks with maximum-likelihood methods[37]	Provides estimates without the need to screen controls; genotype data in relatives can be incorporated but are not required	Is limited by the accuracy of the family history; risks may be overestimated if familial factors are not accounted for	*BRCA1/2*, *PALB2*[10,22]
Segregation in families		Can be applied in families that have been selected for a strong family history; controls are not required	Requires samples on multiple persons from the same family; power is typically very limited	*CHEK2*[30]
Prospective cohort		Provides direct estimates of absolute risk	Requires long-term investment; is prohibitively large except in the case of high-risk variants; risk estimates may altered by interventions (e.g., prophylactic surgery); risk estimates are affected by other familial factors	*BRCA1/2*[13]

evidence that the rate ratio declines with age. The published overall relative risk estimates can thus provide a misleading estimate of lifetime risk. Ideally, age-specific estimates are required, but the data available on risks for older women are often limited.

STUDY DESIGN

Appropriate study design is critical for both the identification of disease-associated alleles and the derivation of reliable risk estimates. Several study designs are available (Table 4). The use of case–control studies for risk estimation involving rare variants can be problematic; family-based methods, including kin–cohort designs and cosegregation analysis, provide alternatives, but these methods also have pitfalls. Furthermore, many studies are based on a few variants that are restricted to specific populations; although it is generally assumed that the risk estimates associated with different truncating variants observed in other populations are similar, it is usually impossible to test this assumption.

OVERESTIMATION OF RISK

The problems of publication bias, in which negative studies are not published, and winner's curse, whereby an initial study identifying an association tends to overestimate the risk, should be noted.[38] Furthermore, many gene-discovery studies oversample for early-onset cases of disease or cases with a family history. This approach improves power but leads to seriously biased risk estimates unless the ascertainment is allowed for in the analysis. Moreover, risk estimates based on data from highly selected families may not reflect the true "average" risk for all carriers of pathogenic variants, because such biased sampling results in a selection of individuals that are not random with respect to other modifiers of risk.

EVIDENCE OF ASSOCIATION FOR SPECIFIC GENES

Here we review several genes for which some evidence of an association with breast cancer has been reported. A summary of the genes for which an association with breast cancer has, in our view, been established is given in Table 3. See Table S2 in the Supplementary Appendix for a list of genes for which an association with breast cancer has been suggested but not established and Table S3 for a summary of the studies used to derive estimates of breast-cancer risk. The Methods section in the Supplementary Appendix summarizes the methods used to derive summary estimates of risk.

BRCA1 AND *BRCA2*

The clinical validity and utility of testing for variants in *BRCA1* and *BRCA2* are well established. There is overwhelming evidence that most protein-truncating variants in these genes are associated with a high risk of breast cancer and other cancers.[10,12,13] Even among protein-truncating variants, however, variant-specific differences in risk have been observed.[39] Furthermore, a polymorphic nonsense variant at the carboxyl terminus of *BRCA2*, p.Lys3326Ter, has been reported to be associated with a relative risk of breast cancer of 1.4 (90% confidence interval [CI], 1.2 to 1.7),[40] which is substantially lower than the risks conferred by more proximal truncating variants (Table 3).

TP53, *CDH1*, *PTEN*, *STK11*, AND *NF1*

Mutations in *TP53*, *CDH1*, *PTEN*, *STK11*, and *NF1* cause pleiotropic tumor syndromes in which breast cancer is only one feature. Germline mutations in *TP53* (both protein-truncating and missense mutations) are responsible for the Li–Fraumeni syndrome, in which carriers are predisposed to childhood sarcomas, brain tumors, adrenocortical carcinoma, and other rare cancers, in addition to breast cancer.[41] Although the association with breast cancer is not controversial, reliable estimates of risk are lacking; most studies are based on pedigrees in which family members have features of the Li–Fraumeni syndrome and thus are subject to ascertainment bias. However, a study based on carriers of a *TP53* mutation identified through probands with childhood sarcoma has also reported a high risk of breast cancer.[15] Similar ascertainment biases apply to mutations in *PTEN* and *STK11*. Mutations in *PTEN* are associated with the Cowden syndrome, in which breast cancer is a characteristic of the clinical phenotype,[16,17,42] and mutations in *STK11* are associated with the Peutz–Jeghers syndrome and an increased risk of breast cancer.[19] Protein-truncating variants in *CDH1*, which are known to be associated with diffuse-type gastric cancer, are also thought to be as-

sociated with an increased risk of breast cancer (specifically, the lobular subtype), with a reported relative risk of 6.6 (90% CI, 2.2 to 19.9; P=0.004).[18] Recent cohort studies[20,21] have reported an elevated risk of breast cancer in women with neurofibromatosis type 1 (odds ratio, 2.6; 90% CI, 2.1 to 3.2).

PALB2, *CHEK2*, *ATM*, *NBN*, AND RELATED GENES

There is strong evidence that protein-truncating variants in four other genes involved in DNA repair confer an increased risk of breast cancer. Among these genes, mutations in *PALB2* appear to confer the highest risks. A large family-based study estimated the risk of breast cancer to be approximately six times as high among carriers as compared with noncarriers,[22] although two case–control studies based on the Finnish founder variant, c.1592delT, estimated somewhat lower risks.[23,25] In a meta-analysis of these estimates the combined relative risk was 5.3 (90% CI, 3.0 to 9.4). Thus, although *PALB2* mutations may fall into the high-risk category (in which the risk of cancer is more than four times as high as that in the general population), the confidence limits are too wide to be certain. Most of the data for *CHEK2* relate to the c.1100delC variant, which is found fairly frequently in Northern European populations.[30] On the basis of two large case–control analyses, we calculated an estimated relative risk of breast cancer of 3.0 (90% CI, 2.6 to 3.5).[31,32] Truncating variants in *ATM* have been evaluated in both case–control studies (with selected cases)[26] and cohort studies of relatives of patients with ataxia–telangiectasia.[27-29] In a meta-analysis of the three largest cohort studies of relatives of patients with ataxia–telangiectasia, the estimated relative risk of breast cancer was 2.8 (90% CI, 2.2 to 3.7; $P=4.7\times10^{-11}$), a value similar to that for truncating variants in *CHEK2*.

In *NBN*, one protein-truncating variant, c.657del5, is sufficiently common in some Eastern European populations to allow its evaluation in a case–control study. A meta-analysis of 10 studies reported strong evidence of an association with breast-cancer risk for this variant (summary relative risk, 2.7; 90% CI, 1.9 to 3.7; $P=5\times10^{-7}$).[34] More limited evidence is available for two other DNA-repair genes, *MRE11A* and *RAD50*, which encode proteins that form an evolutionarily conserved complex with *NBN*.[43-48]

Mutations in three other DNA repair genes, *RAD51C*, *RAD51D*, and *BRIP1*, have shown clear evidence of an association with ovarian cancer.[49-53] However, in each case, the evidence for association with breast cancer is limited. Recent exome studies and targeted sequencing studies have suggested that breast cancer is associated with deleterious variants in *FANCC*,[54] *FANCM*,[55] and *XRCC2*.[56] In none of these instances, however, does the evidence reach the threshold level (P<0.0001) that we propose for DNA-repair genes. The recent findings of deleterious mutations in *RECQL* in women with a strong family history of breast cancer, however, suggests that this gene confers susceptibility to breast cancer.[57,58]

OTHER GENES

The panels currently marketed for the prediction of risk of cancer contain many other genes, most of which have been included by virtue of their relevance to rare mendelian cancer syndromes. Variants in some of these genes may also be associated with breast cancer. Mutations in DNA mismatch-repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) may be associated with breast cancer, but in a recent review, Win et al.[59] concluded that the evidence was equivocal. It has also been suggested that *MUTYH* variants that confer a predisposition to polyposis colorectal cancer may confer a predisposition to breast cancer, but a recent case–control study reported no association.[60] Another recent study suggested that carriers of *MEN1* mutations may be at increased risk for breast cancer.[61] A recent case–control study has reported an association between rare variants in *PPM1D* and breast cancer.[62] However, this association does not reach our proposed significance threshold, and, in addition, the sequence variants are observed as mosaics in lymphocytes and are not inherited. There is currently no clear evidence of an association between breast cancer and any other gene.

MISSENSE VARIANTS

With the exception of *TP53*, the assessment of the risk of breast cancer from missense variants is much more problematic than it is for protein-truncating variants. Some missense variants in specific domains of *BRCA1* and *BRCA2* confer high risks of breast and ovarian cancer, but the

great majority do not.[63,64] For these genes, algorithms based on conservation, pedigree data, and analysis of tumor subtype can be used to predict the pathogenicity of some variants.[63,65,66] Similar considerations may apply to *ATM* and *CHEK2* — missense variants falling in key functional domains and at positions that show a high degree of species conservation are more likely to be associated with increased risk.[67] However, even for *BRCA1* and *BRCA2*, the breast-cancer risk associated with the large majority of missense variants remains unknown; such variants are referred to as variants of unknown significance. Moreover, clearly pathogenic missense variants need not be associated with the same risk as truncating variants. For example, the *CHEK2* missense variant p.Ile157Thr confers a lower risk of breast cancer than the *CHEK2* c.1100delC truncating variant,[33] whereas *ATM* p.Val2424Gly appears to be associated with a higher risk of breast cancer than truncating variants (8.0; 90% CI, 2.8 to 22.5; P=0.0005).[68] A more systematic approach to this problem would involve defining risks on the basis of variant classes that are defined through prediction algorithms based on in silico data. However, even though existing data provide good evidence that missense variants falling at highly conserved positions in several genes confer disease risk, and that such variants may make an important contribution to the heritability of breast cancer,[69] no system has been established for use in the classification of variants that would allow such estimates of risk to be used clinically.

RISK MODIFIERS AND ABSOLUTE RISKS

For the purposes of genetic counseling, relative risks need to be converted into absolute risks. For an "average" mutation carrier, absolute risks can be calculated in a straightforward manner by combining the estimated relative risk with population incidence rates. The results are illustrated in Figure 1 for carriers of mutations in *PALB2* and *CHEK2*.

However, the calculation of the absolute risk associated with a given variant must also account for the risk associated with other genetic factors, lifestyle, and family history. There is strong evidence that the absolute risk of breast cancer in carriers of *BRCA1*, *BRCA2*, *PALB2*, and

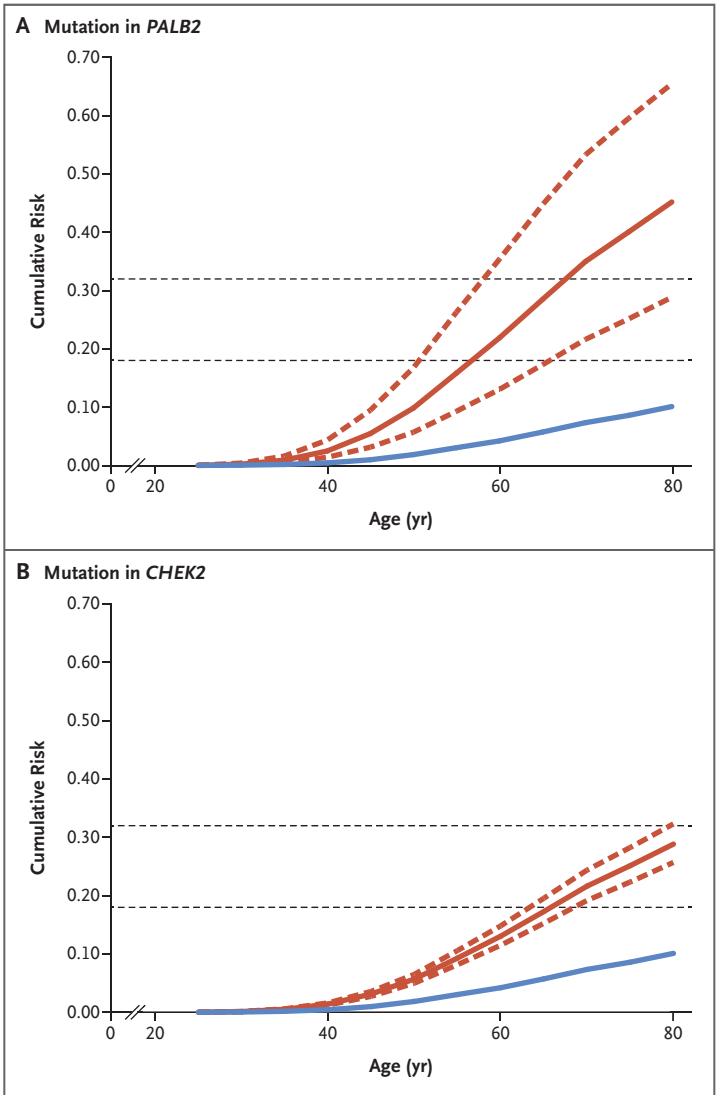

Figure 1. Predicted Cumulative Risk of Breast Cancer for a Carrier of a Deleterious Mutation in *PALB2* and for a Deleterious Mutation in *CHEK2*.

Solid red lines represent summary estimates, and red dashed lines the upper and lower 90% confidence limits. The absolute risks were estimated by applying the estimates of relative risk to the rates for the incidence of breast cancer in England from 2003 through 2007 (obtained from the database Cancer Incidence in Five Continents, volume X).[36] The solid blue lines represent the cumulative risks according to these population incidence rates (i.e., corresponding to a relative risk of 1). Estimates ignore competing mortality (i.e., they represent the cumulative risks in the absence of death from another cause). The dashed horizontal black lines represent lifetime risks that are twice and four times as high as the population average. Thus, a "typical" carrier of the *CHEK2* mutation is likely to fall into the category of moderate risk. The best estimate for carriers of the *PALB2* mutation places them in the high-risk category, but the confidence interval for the estimate is such that their risk may be moderate. These estimates constitute average cumulative risks (for a woman not selected for other risk factors) and are modified by other risk factors, including family history.

CHEK2 mutations is higher among women with a strong family history of breast cancer.[10,22,30,70] It has also been shown that the absolute risk of breast cancer in carriers of *BRCA1* and *BRCA2* mutations depends on the risks associated with their single-nucleotide–polymorphism (SNP) profile.[71] A broader question is that of how the risks associated with genetic variants should be combined with risk factors associated with lifestyle. Several studies indicate that the risks associated with common SNPs and other risk factors combine in a multiplicative rather than an additive fashion,[72-74] and it would be reasonable to assume that rare variants combine with other risk factors in a similar manner. The evidence regarding the combined effects of genetic and lifestyle factors is both limited and conflicting for variants in *BRCA1* and *BRCA2*,[75] and no evidence is available for other genes. In addition, absolute risks need to be adjusted for competing risks in analyses of mortality, a factor that may be important in to our understanding of genes associated with cancers other than breast cancer.

Almost all the available data relate to women of European ancestry. At present, it is unclear whether the available estimates of relative risk can be safely extrapolated to women of other ancestries or to populations with different incidences of breast cancer.

CONCLUSIONS

We have discussed some of the difficulties of assigning risk to rare variants and reviewed the genes for which the evidence of association with breast cancer is sufficiently robust to be incorporated into personalized risk prediction. Variants that are predicted to truncate *BRCA1* and *BRCA2* (together with a subset of missense variants) confer a high risk of breast cancer; *PALB2* and perhaps *PTEN* may also fall in this category, but the evidence is insufficient to place them confidently in the category of high risk rather than moderate risk. For *TP53*, both missense and protein-truncating variants are associated with substantially increased risks of breast cancer. Genes that fall into the category of moderate risk (for which fully deleterious mutations confer a risk of breast cancer that is two to four times as high as that in the general population) include *CHEK2*, *ATM*, and *NF1*. There is clear evidence for an association with risk of cancer for *STK11*, *CDH1*, and *NBN*, but the risk estimates are too imprecise for categorization. Estimates of risk for *PTEN*, *STK11*, and *CDH1* are derived entirely from studies of selected patients identified through specialized clinics and may be seriously overestimated. We found insufficient evidence to establish any other genes as conferring a predisposition for breast cancer and would caution against their use in the prediction of breast-cancer risk. As the costs of sequencing decline, it is inevitable that the use of gene-panel testing, and indeed whole-exome and whole-genome sequencing, will become widespread. Therefore, there is an urgent need for much larger, well-designed population- and family-based studies in diverse populations that will provide reliable estimates of risk for the purpose of counseling. The systematic collection of data from ongoing use of panel testing linked to the epidemiologic and clinical data may also make an important contribution. Other genes that convey susceptibility to breast cancer (and perhaps rarer variants in noncoding sequences) will probably be identified and may be added to genetic-testing panels. Panel testing can make a useful contribution to prediction of a woman's risk of breast cancer, but end users need to be aware of the limitations of these panels.

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1. Assoc. for Molecular Pathology v. Myriad Genetics, Inc. 569 U.S. ___ (2013) (https://supreme.justia.com/cases/federal/us/569/12-398).
2. Grady D, Pollack A. Finding risks, not answers, in gene tests. New York Times, September 22, 2014 (http://www.nytimes.com/2014/09/23/health/finding-risks-not-answers-in-gene-tests.html?module=Search&mabReward=relbias%3Ar%2C{%222%22%3A%22RI%3A13%22}&_r=0).
3. Haddow JE, Palomaki GE. ACCE: a model process for evaluating data on emerging genetic tests. In: Khoury MJ, Little J, Burke W, eds. Human genome epidemiology: a scientific foundation for using genetic information to improve health and prevent disease. Oxford, United Kingdom: Oxford University Press, 2003:217-33.
4. National Institute for Health and Care Excellence. Familial breast cancer: classification and care of people at risk of familial breast cancer and management of breast cancer and related risks in people with a family history of breast cancer. London: National Collaborating Center for Cancer, 2013 (http://www.cancer.gov/types/breast/hp/breast-ovarian-genetics-pdq#section/all).
5. Hayes DF, Allen J, Compton C, et al. Breaking a vicious cycle. Sci Transl Med 2013;5:cm6.
6. Sharfstein J. FDA regulation of laboratory-developed diagnostic tests: protect the public, advance the science. JAMA 2015;313:667-8.
7. Lander ES. Cutting the Gordian helix — regulating genomic testing in the era of precision medicine. N Engl J Med 2015;372:1185-6.
8. Evans BJ, Burke W, Jarvik GP. The FDA and genomic tests — getting regulation right. N Engl J Med 2015;372:2258-64.
9. Hollestelle A, Wasielewski M, Martens JW, Schutte M. Discovering moderate-risk breast cancer susceptibility genes. Curr Opin Genet Dev 2010;20:268-76.
10. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-30.
11. Lee AJ, Cunningham AP, Kuchenbaecker KB, Mavaddat N, Easton DF, Antoniou AC. BOADICEA breast cancer risk prediction model: updates to cancer incidences, tumour pathology and Web interface. Br J Cancer 2014;110:535-45.
12. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 2007;25:1329-33.
13. Mavaddat N, Peock S, Frost D, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst 2013;105:812-22.
14. Hisada M, Garber JE, Fung CY, Fraumeni JF Jr, Li FP. Multiple primary cancers in families with Li-Fraumeni syndrome. J Natl Cancer Inst 1998;90:606-11.
15. Hwang SJ, Lozano G, Amos CI, Strong LC. Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. Am J Hum Genet 2003;72:975-83.
16. Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. J Med Genet 2013;50:255-63.
17. Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 2012;18:400-7.
18. Pharoah PD, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 2001;121:1348-53.
19. Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12:3209-15.
20. Madanikia SA, Bergner A, Ye X, Blakeley JO. Increased risk of breast cancer in women with NF1. Am J Med Genet A 2012;158A:3056-60.
21. Seminog OO, Goldacre MJ. Age-specific risk of breast cancer in women with neurofibromatosis type 1. Br J Cancer 2015;112:1546-8.
22. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in *PALB2*. N Engl J Med 2014;371:497-506.
23. Heikkinen T, Kärkkäinen H, Aaltonen K, et al. The breast cancer susceptibility mutation PALB2 1592delT is associated with an aggressive tumor phenotype. Clin Cancer Res 2009;15:3214-22.
24. Rahman N, Seal S, Thompson D, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 2007;39:165-7.
25. Erkko H, Xia B, Nikkilä J, et al. A recurrent mutation in PALB2 in Finnish cancer families. Nature 2007;446:316-9.
26. Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet 2006;38:873-5.
27. Thompson D, Duedal S, Kirner J, et al. Cancer risks and mortality in heterozygous ATM mutation carriers. J Natl Cancer Inst 2005;97:813-22.
28. Janin N, Andrieu N, Ossian K, et al. Breast cancer risk in ataxia telangiectasia (AT) heterozygotes: haplotype study in French AT families. Br J Cancer 1999;80:1042-5.
29. Olsen JH, Hahnemann JM, Børresen-Dale AL, et al. Breast and other cancers in 1445 blood relatives of 75 Nordic patients with ataxia telangiectasia. Br J Cancer 2005;93:260-5.
30. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002;31:55-9.
31. CHEK2 Breast Cancer Case–Control Consortium. CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from nine studies. Am J Hum Genet 2004;74:1175-82.
32. Weischer M, Nordestgaard BG, Pharoah P, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. J Clin Oncol 2012;30:4308-16.
33. Kilpivaara O, Vahteristo P, Falck J, et al. CHEK2 variant

I157T may be associated with increased breast cancer risk. Int J Cancer 2004;111:543-7.
34. Zhang G, Zeng Y, Liu Z, Wei W. Significant association between Nijmegen breakage syndrome 1 657del5 polymorphism and breast cancer risk. Tumour Biol 2013;34:2753-7.
35. Hogervorst FB, Nederlof PM, Gille JJ, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. Cancer Res 2003;63:1449-53.
36. Cancer incidence in five continents. Vol. X. Lyon, France: International Agency for Research on Cancer, 2013 (http://www.iarc.fr/en/publications/pdfs-online/epi/sp164/index.php).
37. Easton DF, Hopper JL, Thomas DC, et al. Breast cancer risks for BRCA1/2 carriers. Science 2004;306:2187-91.
38. Kraft P. Curses — winner's and otherwise — in genetic epidemiology. Epidemiology 2008;19:649-51.
39. Thompson D, Easton D. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. Am J Hum Genet 2001;68:410-9.
40. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45:353-61.
41. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-8.
42. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16:(1)64-7.
43. Heikkinen K, Rapakko K, Karppinen SM, et al. RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 2006;27:1593-9.
44. Tommiska J, Seal S, Renwick A, et al. Evaluation of RAD50 in familial breast cancer predisposition. Int J Cancer 2006;118:2911-6.
45. Mosor M, Ziółkowska-Suchanek I, Roznowski K, Baranowska M, Januszkiewicz-Lewandowska D, Nowak J. RAD50 gene mutations are not likely a risk factor for breast cancer in Poland. Breast Cancer Res Treat 2010;123:607-9.
46. He M, Di GH, Cao AY, et al. RAD50 and NBS1 are not likely to be susceptibility genes in Chinese non-BRCA1/2 hereditary breast cancer. Breast Cancer Res Treat 2012;133:111-6.
47. Bartkova J, Tommiska J, Oplustilova L, et al. Aberrations of the MRE11-RAD50-NBS1 DNA damage sensor complex in human breast cancer: MRE11 as a candidate familial cancer-predisposing gene. Mol Oncol 2008;2:296-316.
48. Damiola F, Pertesi M, Oliver J, et al. Rare key functional domain missense substitutions in MRE11A, RAD50, and NBN contribute to breast cancer susceptibility: results from a Breast Cancer Family Registry case-control mutation-screening study. Breast Cancer Res 2014;16:R58.
49. Pelttari LM, Heikkinen T, Thompson D, et al. RAD51C is a susceptibility gene for ovarian cancer. Hum Mol Genet 2011;20:3278-88.
50. Loveday C, Turnbull C, Ruark E, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet 2012;44:475-6.
51. Loveday C, Turnbull C, Ramsay E, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet 2011;43:879-82.
52. Pelttari LM, Kiiski J, Nurminen R, et al. A Finnish founder mutation in RAD51D: analysis in breast, ovarian, prostate, and colorectal cancer. J Med Genet 2012;49:429-32.
53. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet 2011;43:1104-7.
54. Thompson ER, Doyle MA, Ryland GL, et al. Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. PLoS Genet 2012;8(9):e1002894.
55. Kiiski JI, Pelttari LM, Khan S, et al. Exome sequencing identifies FANCM as a susceptibility gene for triple-negative breast cancer. Proc Natl Acad Sci U S A 2014;111:15172-7.
56. Park DJ, Lesueur F, Nguyen-Dumont T, et al. Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet 2012;90:734-9.
57. Cybulski C, Carrot-Zhang J, Kluźniak W, et al. Germline *RECQL* mutations are associated with breast cancer susceptibility. Nat Genet 2015; April 27 (Epub ahead of print).
58. Sun J, Wang Y, Xia Y, et al. Mutations in *RECQL* gene are associated with predisposition to breast cancer. PLoS Genet 2015;11(5):e1005228.
59. Win AK, Lindor NM, Jenkins MA. Risk of breast cancer in Lynch syndrome: a systematic review. Breast Cancer Res 2013;15:R27.
60. Out AA, Wasielewski M, Huijts PE, et al. MUTYH gene variants and breast cancer in a Dutch case–control study. Breast Cancer Res Treat 2012;134:219-27.
61. Dreijerink KM, Goudet P, Burgess JR, Valk GD. Breast-cancer predisposition in multiple endocrine neoplasia type 1. N Engl J Med 2014;371:583-4.
62. Ruark E, Snape K, Humburg P, et al. Mosaic PPM1D mutations are associated with predisposition to breast and ovarian cancer. Nature 2013;493:406-10.
63. Easton DF, Deffenbaugh AM, Pruss D, et al. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. Am J Hum Genet 2007;81:873-83.
64. Lindor NM, Guidugli L, Wang X, et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). Hum Mutat 2012;33:8-21.
65. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. Am J Hum Genet 2004;75:535-44.
66. Plon SE, Eccles DM, Easton D, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 2008;29:1282-91.
67. Le Calvez-Kelm F, Lesueur F, Damiola F, et al. Rare, evolutionarily unlikely missense substitutions in CHEK2 contribute to breast cancer susceptibility: results from a breast cancer family registry case-control mutation-screening study. Breast Cancer Res 2011;13:R6.
68. Goldgar DE, Healey S, Dowty JG, et al. Rare variants in the ATM gene and risk of breast cancer. Breast Cancer Res 2011;13:R73.
69. Tavtigian SV, Chenevix-Trench G. Growing recognition of the role for rare missense substitutions in breast cancer susceptibility. Biomark Med 2014;8:589-603.
70. Johnson N, Fletcher O, Naceur-Lombardelli C, dos Santos Silva I, Ashworth A, Peto J. Interaction between CHEK2*1100delC and other low-penetrance breast-cancer susceptibility genes: a familial study. Lancet 2005;366:1554-7.
71. Antoniou AC, Spurdle AB, Sinilnikova OM, et al. Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. Am J Hum Genet 2008;82:937-48.
72. Nickels S, Truong T, Hein R, et al. Evidence of gene-environ-

ment interactions between common breast cancer susceptibility loci and established environmental risk factors. PLoS Genet 2013;9(3):e1003284.
73. Campa D, Kaaks R, Le Marchand L, et al. Interactions between genetic variants and breast cancer risk factors in the Breast and Prostate Cancer Cohort Consortium. J Natl Cancer Inst 2011;103:1252-63.
74. Rudolph A, Milne RL, Truong T, et al. Investigation of gene-environment interactions between 47 newly identified breast cancer susceptibility loci and environmental risk factors. Int J Cancer 2015;136(6):E685-E696.
75. Friebel TM, Domchek SM, Rebbeck TR. Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju091.
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